IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of:	)	
	)	
BAO et al.	)	Group Art Unit: 1634
	)	
Application Serial No.: 10/729,619	)	Examiner: Whisenant, Ethan C.
	)	
Filing Date: December 5, 2003	)	Confirmation No.: 2934
	)	
For: TREATMENT OF SUBSTRATES FOR IMMOBILIZING BIOMOLECULES	)	
	)	
	)	

DECLARATION UNDER 37 C.F.R. § 1.131

Mail Stop Amendment
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

GARDNER GROFF
SANTOS & GREENWALD, P.C.
Customer Number 23506

Sir:

I declare that:

1. The effective filing date of U.S. Patent Publication No. 2005/0059137 to Lee ("the Lee publication") under 35 U.S.C. § 102(e) is May 31, 2001.

2. Attached in Exhibit A is a copy of an invention disclosure report. In the report, it is shown that treatment of arrays with sodium borohydride followed by hybridization results in the elimination of autofluorescence.

3. Based on the evidence presented herein, the invention was conceived and reduced to practice in the United States as recited in U.S. application serial no. 10/729,619 prior to May 31, 2001.

4. I further declare that all statements made herein of my knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine, imprisonment, or both, under U.S.C. Title 18, § 1001, and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

DATED: July 5, 2006

Thomas R. Beall
Authorized Attorney for Assignee

B. Problem Solved

We have successfully demonstrated that sodium borohydride (0.25%) treatment prior to hybridization of the labeled probes results in significant elimination of background/auto-fluorescence on corning cDNA and oligo arrays. This in turn resulted in distinct improvement in the quality of fluorescing signals thereby increasing the sensitivity of detection. In addition, this treatment with sodium borohydride did neither affect DNA retention on the slide nor the hybridization efficiency of target to the probe as evidenced by the hybridization results.

This technique is rapid, reproducible and easily applicable for reducing auto-fluorescence, and can be used in single and dual color applications on DNA, Oligo and Protein/Antibody/Peptide arrays.

C. Detailed Description of Invention

Method for labeling and hybridization

Reagents. Human RNA (Clonetech); Superscript II reverse transcriptase, DTT, RNAse H, formamide,); random 6mers, human Cot 1 DNA (Life Technologies); Cy3-dCTP, Cy5-dCTP, (Perkin Elmer), RNAse A (USB), QIAquick PCR purification kit (Qiagen); and bovine serum albumin (BSA), dextran sulfate, nuclease-free water, and poly A (Sigma) were all used as received.

Procedure:

I Scan microarrays to obtain auto-fluorescent signal before $NaBH_4$ treatment. A GenePix 4000A (Axon Instruments) fluorescence scanner was used to obtain the Cy3/Cy5 fluorescence images using a PMT setting of 700-950 volts. All images were analyzed using GenePix Pro 3.0 analysis software (Axon Instruments).

II NaBH4 treatment:

1. Freshly made pre-hybridization solution: 2X SSC/0.05% SDS/0.2% BSA (use fresh made BSA).
2. Add 100 ml of pre-hyb solution into a Coplin jar and warm up solution to 42°C in water bath (takes about 20-30 min) and soak slides (max. 4 slides/jar) for 10 min, at 42°C.
3. Transfer slides to Coplin jar filled with 2X SSC/0.05% SDS/ 0.25% $NaBH_4$ at 42°C for 15 min.
4. Transfer slides to Coplin jar filled with 1X SSC at RT for 2 min.
5. Transfer slides to Coplin jar filled with 0.2X SSC at RT for 2 min.

6. Repeat step 5 twice
7. Dry slides by spinning at 2000 rpm, 2min, 25°C.

III Scan microarrays again to examine the elimination of auto-fluorescence.

RNA Labeling. A 22 μL solution containing 2μg human poly A RNA and 2.5 μg random 6mers was incubated for 5 minutes at 70° C, briefly chilled on ice and then added to a 20μL solution containing i) 8μL 5X Superscript II buffer; ii) 4μL 100mM DTT, iii)2μL of a dNTP mixture consisting of 10mM each dGTP, dATP, dTTP, and 1mM of dCTP, iv) 2μL Cy3 or Cy5 dCTP (1mM), and vi) 2μL reverse transcriptase. This 40μL reaction mixture was incubated for 10 minutes at room temperature followed by an hour incubation at 42° C. After addition of 1μL of reverse transcriptase, the mixture was incubated for an additional hour at 42° C. RNA was degraded by the addition of 1μL RNase H and 0.25μL RNase A followed by incubation for 15 minutes at 37° C. Probes were purified using a QIAquick PCR purification kit according to the manufacturer's instructions. The cDNA concentration and the amount of Cy3/Cy5 incorporation was measured on an Agilent 8453E UV-Vis spectrophotometer.

Hybridization.
Each array was hybridized with a solution consisting of 29% formamide, 2.25X SSC, 6% dextran sulfate, 0.17μg/μL poly A, 0.10μg/μL Cot 1 DNA, 0.2% BSA, and a given amount of labeled cDNA. For hybridization, 60μL of this solution was spotted onto the array and then spread over the entire surface using a 24mm x 60 mm coverslip. The arrays were incubated overnight at 42° C.

Results:

A remarkable success in the elimination of auto-fluorescence was achieved with sodium borohydride treatment.

1. Fig.1 shows the images of the arrays before and after treatment with 0.25% sodium borohydride. Analysis of the images showed that the average signal intensity due to auto-fluorescence before treatment was 3196+/- 2486 which upon washing with the prehyb solution was reduced to 840 +/- 249. When sodium borohydride was added to the prehyb solution, the signal intensity was reduced to 100 +/- 42. A similar trend was observed in background signal intensity.
2. Fig.2 shows the reduction of auto-fluorescence in cancer arrays. When the reduction process with sodium borohydride was extended to 30 min greater elimination of auto-fluorescence was achieved as can be clearly seen from the figure.
3. Similarly, the elimination of auto-fluorescence on oligo arrays was quite significant with 0.25% sodium borohydride as depicted in Fig.3

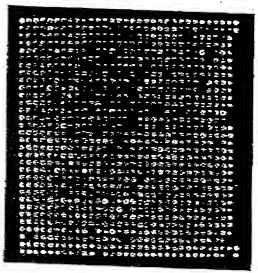
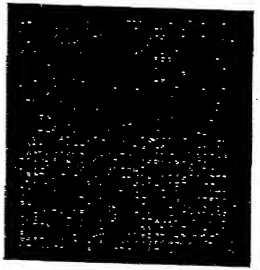
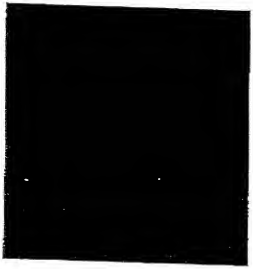
Hybridization results:

1. Hybridization of human 6K arrays (treated and untreated with sodium borohydride) with 0.25 μg Cy3, Cy5 labeled probes from human brain total RNA showed that in untreated slides the Cy3 auto-fluorescence significantly affected the signal to noise

ratio in comparison to slides treated with 0.25% sodium borohydride. This ultimately led to improvement in gene expression profile. These results are clearly depicted in Fig. 4.

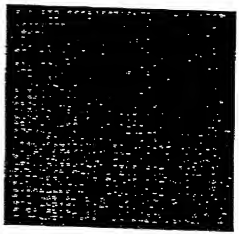
2. Fig. 5 shows the self-self hybridization on 4k cancer arrays after treatment with sodium borohydride. Data analysis showed an excellent correlation of signal intensities in both Cy3 and 5 channels. The dynamic range of detection was greater than 2.5 logs. The gene expression ratio was found to be in the range of 0.5-1.5 which is well within the statistically accepted range. The expression profile of genes was quite consistent on duplicate arrays printed on the same slide.
3. Hybridization on Oligo arrays also showed improved signal /noise ratio after treatment with sodium borohydride in comparison to the untreated arrays as can be seen from Fig. 6.

Reduction of Auto-Fluorescence and Cy3 Background

Before Treatment	After Pre-Hyb without Reducing reagent	After Pre-Hyb with reducing reagent
		
Median Cy3 RFU	Median Cy3 RFU	Median Cy3 RFU
Spot: 3196 +/- 2486 Bgd: 237 +/- 56	Spot: 840 +/- 249 Bgd: 264 +/- 37	Spot: 100 +/- 42 Bgd: 66 +/- 5

Human 6K (Sub-Grid A1) Scanned at PMT 800

Auto-fluorescence Reduction with Reducing Reagent on Cancer Array



Without NaBH4

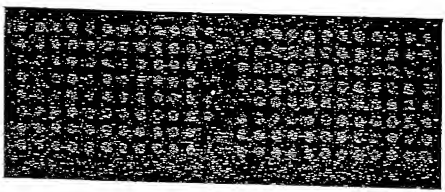
With NaBH4 -20 min

With NaBH4-10 min.

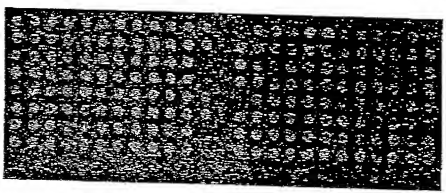
With NaBH4-30 min.

Oligo Array

Before Pre-wash

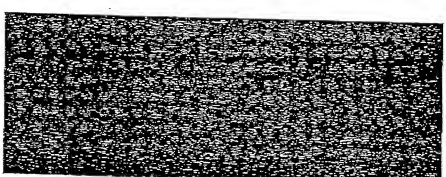


Slide #1204368

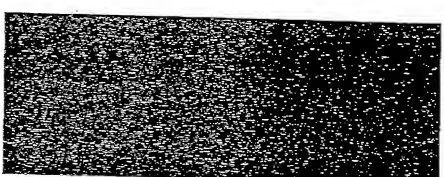


Slide #1204366

After Pre-wash



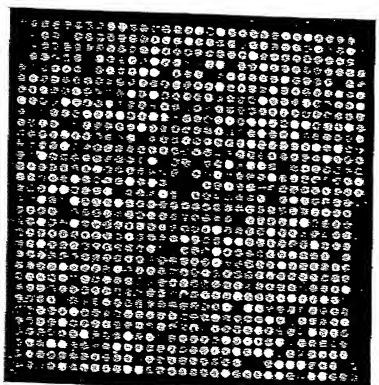
Without NaBH4



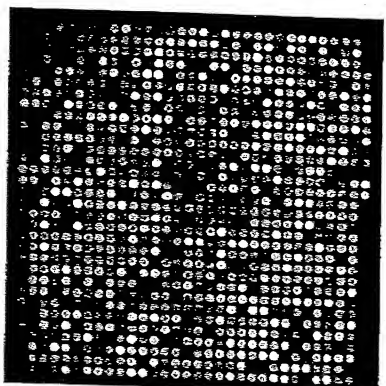
With NaBH4

Conclusion:NaBH4 treatment significantly reduces oligo array (targets and GAPS surface) auto-fluorescence.

Human 6k array with Human Brain RNA Probe Self-Self Hybridization (Sub-Grid 5)



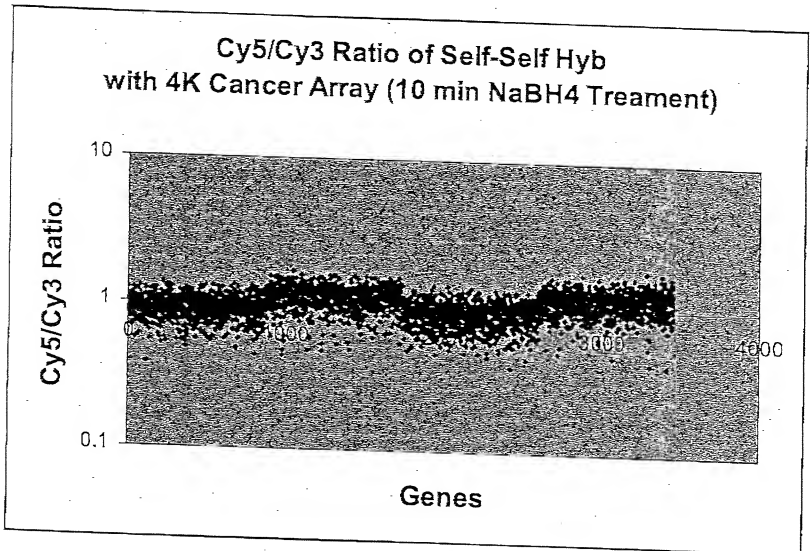
Without NaBH4 Treatment



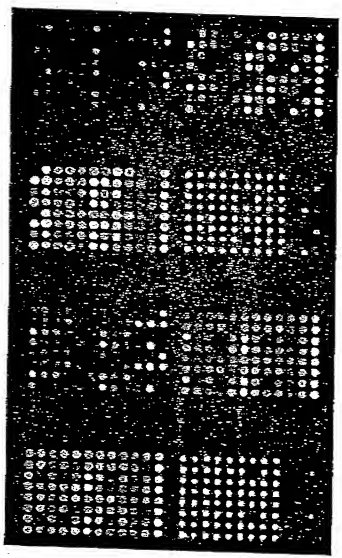
With NaBH4 Treatment

Results:

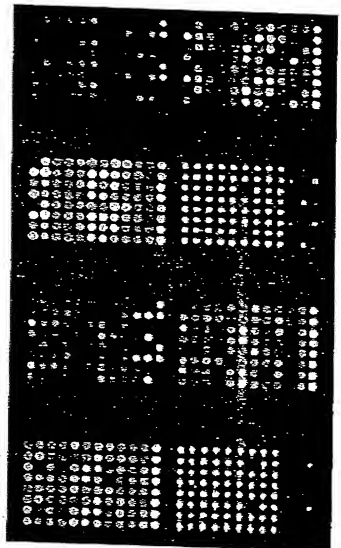
1. Cy3 auto-fluorescence affects signal ratio in the array without NABH4 treatment.
2. NaBH4 treatment improved gene expression profiling.


Cy5/Cy3 Ratio of Self-Self Hyb
with 4K Cancer Array (10 min NaBH4 Treament)
Cy5/Cy3 Ratio
10
1
0.1
0
1000
3000
4000
Genes

Liver + Bacterial Probe Hybridization on Oligo Array



Without NaBH4 prewash



With NaBH4 prewash